Title: Method Validation Report for the Elecsys Anti-SARS-CoV-2 Assay

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Pfizer Vaccine Research and Development
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Title: Method Validation Report for the Elecsys Anti-SARS-CoV-2 Assay

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SYNOPSIS

The Roche Elecsys Anti-SARS-CoV-2 assay is an FDA emergency use authorized qualitative test for detection of antibodies to SARS-CoV-2 in human serum and plasma. The assay, performed on the Roche Cobas® platform, detects antibodies to the SARS-CoV-2 N protein in serum and plasma samples. This document describes the validation results of this assay and confirms that the assay is fit for its intended use in epidemiological or clinical trials designed to evaluate the efficacy of Pfizer's SARS-CoV-2 vaccine candidate. Assay sensitivity and specificity were evaluated and are comparable to those observed by Roche Diagnostics and other laboratories. Overall assay performance is summarized in this validation report.

Key Validation Outcomes

Clinical Performance Parameter	Percent Agreement (%)	95% One-Sided Lower Confidence Limit (%)
Negative Percent Agreement to pre-COVID-19 serum samples (Clinical Specificity)	100.00	97.72
Positive Percent Agreement to human COVID-19 (b) (4) serum samples (Clinical Sensitivity)	93.50	89.86

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Study Number: N/A

Functional Area: Vaccine Research and Development

Test Facility: Pfizer Vaccine Research, 401 North Middletown Road, Pearl River, NY 10965

Study/Testing Initiation Date: 26 July 2020

Study/Testing Completion Date: 26 July 2020

1. OBJECTIVES

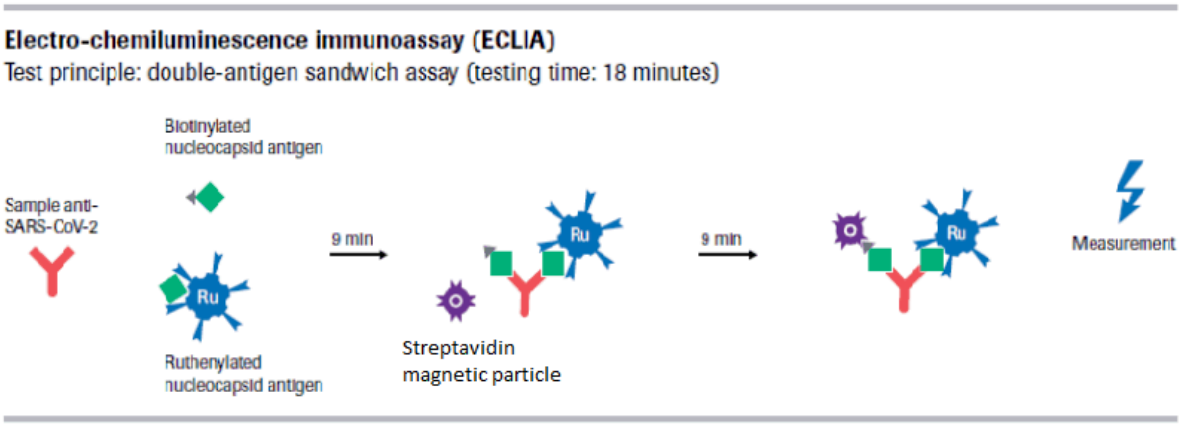
This report summarizes the results for the validation of the FDA emergency use authorization (EUA)-approved Elecsys Anti-SARS-CoV-2 assay as performed on the Roche Cobas® instrument (Cobas® (b) (4) analyzer) for its intended use in clinical trials designed to evaluate the efficacy of Pfizer's SARS-CoV-2 vaccine candidate or other epidemiological studies. Performance parameters, including clinical sensitivity and clinical specificity, were evaluated, as well as overall assay performance. This validation provides documented evidence that the Elecsys Anti-SARS-CoV-2 assay, when performed in accordance with the test method and standard operating procedures (SOPs) by qualified personnel, is suitable for its intended use.

2. INTRODUCTION

The Roche Elecsys Anti-SARS-CoV-2 assay is currently being marketed under an EUA for detecting the presence of antibodies to the N protein of SARS-CoV-2 (antigen not incorporated in Pfizer's SARS-CoV-2 vaccine candidate). The performance characteristics of the Elecsys Anti-SARS-CoV-2 assay are described in the package Instructions for Use (IFU).[1] In addition, a correspondence (VR-MVR-10081-ATT02)[2] from Roche Diagnostics (Indianapolis, IN) is provided in support of this method validation.

The Pfizer assay Test Method, VR-TM-10304,[3] describes the laboratory method for the detection of anti-SARS-CoV-2 N protein antibodies in serum or plasma using the FDA EUA Elecsys Anti-SARS-CoV-2 assay (Roche Diagnostics, Indianapolis, IN). The Elecsys Anti-SARS-CoV-2 assay is a rapid, automated in vitro diagnostic test for the qualitative detection of SARS-CoV-2 N protein specific antibodies in serum or plasma samples. The assay is marketed as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, which would indicate a recent or prior infection. Detection of N protein antibodies to SARS-CoV-2 is carried out by a two-step immunoassay method where biotin and ruthenium N protein conjugates are incubated with human serum or plasma. Streptavidin coated paramagnetic beads are then added in the second step to isolate N protein/antibody complexes and chemiluminescent emissions are measured by a photomultiplier (Figure 1).

Figure 1. Overview of the Elecsys Anti-SARS-CoV-2 Assay



The Elecsys Anti-SARS-CoV-2 assay has been evaluated by other laboratories[4] and demonstrated comparable clinical sensitivity (86.1%, CI 76.5-92.8; n=79 samples) in samples obtained ≥14 days post-COVID-19 diagnosis and clinical specificity (100%) to that described in Roche's IFU[1] and EUA (EUA200514/A001). Although sensitivity was lower in that evaluation compared to Roche's IFU[1] claims (99.5%), the above report used a different sample set of smaller size. However, specificity in the smaller cohort of subjects (n=472) tested in the evaluation described above was highly comparable to Roche's reported specificity (99.8%), which used a much larger cohort of negative samples (n=10,453).

This method validation report successfully fulfills the objectives of the validation protocol VR-MVP-10075[5] and demonstrates that the assay, when performed according to the manufacturer's instructions in Pearl River, NY (b) (4), is suitable for use in clinical trials designed to evaluate the efficacy of Pfizer's SARS-CoV-2 vaccine candidate or other epidemiological studies.

3. GLOSSARY

Table 1. Terms and Definitions

Term	Definition
ACOV2 Cal1	Negative calibrator 1
ACOV2 Cal2	Positive calibrator 2
Coated Microspheres	Streptavidin coated beads at 0.72mg/mL
COI	Cutoff Index
COVID-19	Coronavirus Disease 2019
Elecsys Anti-SARS-CoV-2	An immunoassay intended for qualitative detection of antibodies to SARS-CoV-2 in human serum and plasma
EUA	Emergency Use Authorization
IFU	Instructions for Use
LIMS	Laboratory Information Management System
OHW	Occupational Health and Wellness

Table 1. Terms and Definitions

Term	Definition
QCS	Quality Control Samples
RGR	Reagent Qualification Report
Roche Cobas® Instrument	A fully automated analyzer that uses a patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis (ie, Cobas® (b) (4))
Room Temperature (RT)	Room temperature in this SOP is defined as 20°C with a range of 18-25°C.
SARS	Severe Acute Respiratory Syndrome
SOP	Standard Operating Procedure
SM	Sample Management
TM	Test Method
VPL	Validation Plan
VRD	Vaccine Research and Development

4. MATERIALS AND METHODS

4.1. Materials

Unique reagents obtained for this validation are listed in Supportive Table 11.1, and all other materials used are described in the SOPs referenced in Section 10. Specific details regarding reagent catalog and lot numbers and expiration dates, as well as the instrument identification numbers and maintenance details are captured by the Roche Cobas® system software or documented in the assay worksheets within data packages or electronically stored in LIMS. All other reagents and supplies are described in VR-TM-10304.[3]

4.2. Critical Reagents

The critical reagents, including quality control samples (QCS), used in this assay validation are listed in Supportive Table 11.1. Panel samples used in this validation are described in Supportive Tables 11.2 and 11.3. Briefly, (b) (4) (b) (4) serum samples obtained between(b) (4) days post-PCR diagnosis were received from (b) (4) (b) (4) and used to assess clinical sensitivity. Clinical specificity was assessed with (b) (4) negative serum samples from blood draws that pre-date COVID-19 (b) (4) and were obtained from (b) (4) (b) (4) (b) (4)

4.3. Method

The Elecsys Anti-SARS-CoV-2 assay is an FDA EUA approved assay for the rapid detection of SARS-CoV-2 antibody in serum or plasma. The test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The assay was performed according to the manufacturer's instructions.[1] All critical reagents are provided in Roche's assay kit.

Test Method VR-TM-10304[3] describes the procedures to perform the Elecsys Anti-SARS-CoV-2 assay on serum samples. Barcoded samples were processed for testing as follows:

1. Analyzer was calibrated. Refer to VR-TM-10304[3] for calibration procedure.
2. Patient serum samples were thawed at RT for 20 ± 10 minutes.
3. Sample barcodes were scanned and assigned an Anti-SARS-CoV-2 test.
4. Sample vials were uncapped and placed in the appropriate rack.
5. Racks were placed on the analyzer.
6. The analyzer was started.
7. When the assay was finished, raw data (.csv file) was extracted for analysis.

4.4. Qualified Equipment used for the Method Validation

Table 2. Qualified Equipment used for the Method Validation

Description	Manufacturer	Model #	Equipment ID #	Calibration Date	Expiration Date
cobas (b) (4) analyzer	Roche	(b) (4)	(b) (4)	Jul-20	Jul-21
4C Cold Room	(b) (4)	(b) (4)	(b) (4)	Dec-19	Dec-20
-80C Freezer	(b) (4)	(b) (4)	(b) (4)	Sep-19	Sep-20

4.4.1. Data Handling

4.4.1.1. Data Processing and Output via Instrument Software

1. Data results were presented in qualitative "Positive" or "Negative" and cutoff index (COI) value formats. The results were interpreted automatically by the Roche Cobas® software and shown in the "View Results" window. The Elecsys Anti-SARS-CoV-2 assay provides positive/negative test results based on N specific antibody levels above or below a predetermined COI (Table 3).

Table 3. Cobas® Elecsys Anti-SARS-CoV-2 Result Options

Result Text	Sample COI Value	Negative Control	Positive Control
SARS-CoV-2 Positive	≥1.0	<1.0	≥1.0
SARS-CoV-2 Negative	<1.0	<1.0	≥1.0
INVALID	N/A	>1.0	<1.0
INVALID[a]	Instrument error	Instrument error	Instrument error

a. (b) (4) different error codes are possible on the Roche Cobas® (b) (4) system

For testing validity, the Elecsys Anti-SARS-CoV-2 assay must have both positive and negative controls that pass acceptance criteria at the beginning and end of an assay run. An assay run involves testing [redacted] racks of samples. A rack will hold [redacted] sample vials, which may include calibrators and/or controls.

2. Numerical data in the form of COI values were captured for each sample, calibrator and control. The COI values were used to determine descriptive statistics only.

3. Two controls were tested with every assay run:

   a. Negative Control (Neg) – Ensures that the assay is returning results consistent with a known SARS-CoV-2 antibody negative sample. The negative control passes if it produces a "negative" result. The negative control was tested in the beginning and end of the assay run. Both sets of controls must pass to validate the sample results within the run.

   b. Positive control (Pos) – Ensures that the assay is returning results consistent with a known SARS-CoV-2 antibody positive samples. The positive control passes if it produces a "positive" result. The positive control was tested in the beginning and end of the assay run. Both sets of controls must pass to validate the sample results within the run.

4. As described per the validation protocol VR-MVP-10075,[5] failed tests and samples were repeated as required, per VR-SOP-LC-11296.[6] A failed test is a sample that does not generate a reportable result.

5. EXPERIMENTAL OUTLINE

The validation of the Elecsys SARS-CoV-2 assay was performed as described in VR-MVP-10075.[5] The critical reagents, including quality control samples (QCS), used in this assay validation are listed in Supportive Table 11.1. Formulation of the QCS are documented in VR-RGR-QC-10753.[7] The human serum samples used in this validation are listed in Supportive Tables 11.2 and 11.3.

Specific details regarding reagent catalog and lot numbers and expiration dates, as well as the instrument identification numbers and maintenance details is captured by the Roche Cobas® system software or documented in the assay worksheets within data packages or electronically stored in LIMS. All other reagents and supplies are described in VR-TM-10304.[3]

6. RESULTS AND DISCUSSION

Attachments in support of this validation are described in Table 4. Clinical sensitivity, and specificity data used for the validation analyses described herein are listed in VR-MVR-10081-ATT01 (see Section 9). Roche provided correspondence to the FDA in support of this method validation that is provided in VR-MVR-10081-ATT02.[2]

Table 4. List of Attachments

Name	Content
VR-MVR-10081-ATT01 (see Section 9)	Clinical sensitivity and clinical specificity data listings
VR-MVR-10081-ATT02[2]	Roche correspondence to FDA
VR-MVR-10081-ATT03[8]	Roles and responsibilities for the method validation

6.1. Clinical Specificity / Test Agreement Evaluation

6.1.1. Clinical Specificity / Test Agreement Evaluation Study Design

The specificity of the Elecsys Anti-SARS-CoV-2 assay was evaluated using negative (pre-COVID-19) serum specimens described in Supportive Table 11.3. (b) (4) (b) (4) negative serum samples were tested in the Elecsys Anti-SARS-CoV-2 assay. Clinical serum samples were obtained from (b) (4) (b) (4) (b) (4) The serum specimens were from blood draws that pre-date COVID-19 (b) (4) Though this method validation did not evaluate specificity to other known coronaviruses, such a study was performed by Roche and demonstrated that the Elecsys Anti-SARS-CoV-2 assay was not reactive with non-SARS-CoV-2 coronaviruses (ie, HKU1, NL63, 229E or OC43).[1]

Results are reported as the number of concordant negative results out of the total number of samples tested.

6.1.2. Clinical Specificity / Test Agreement Evaluation Analysis

The Negative Percent Agreement (NPA) was defined as the percentage of the negative samples confirmed by the assay:

NPA = 100* Number of Agreed Negatives/Total Number of Negatives Samples with Valid Results.

The one-sided lower 95% confidence bound for NPA was determined by the exact (Clopper-Pearson) method.

6.1.3. Acceptance Criteria

Results are descriptive in nature and compared to results provided in Roche's IFU[1] and EUA.

6.1.4. Clinical Specificity / Test Agreement Evaluation Results

A summary of the Elecsys anti-SARS-CoV-2 assay testing on negative serum samples that pre-date COVID-19 are shown in Table 5. The assay was 100% specific (n=(b) (4)) in not identifying SARS-CoV-2 antibodies in pre-COVID-19 pandemic samples.

Table 5. Negative Agreement in Serum Samples Obtained Prior to COVID-19

Clinical Performance	Frequency of Elecsys Anti-SARS-CoV-2 Negative Samples	Percent Agreement	Total Samples Tested	95% One-Sided Lower Confidence Limit (%)
Negative Percent Agreement	(b) (4)	100.00	(b) (4)	97.72

6.2. Clinical Sensitivity / Test Agreement Evaluation

6.2.1. Clinical Sensitivity / Test Agreement Evaluation Study Design

The sensitivity of the Elecsys Anti-SARS-CoV-2 assay was evaluated using (b) (4) sera from individuals with a PCR confirmed COVID-19 diagnosis. All (b) (4) serum samples were obtained (b) (4) days post-PCR diagnosis. (b) (4) samples were tested in the Elecsys Anti-SARS-CoV-2 assay and were obtained from (b) (4) (b) (4). The sample IDs are listed in Supportive Table 11.2.

Results are reported as the number of concordant positive results out of the total number of samples tested.

6.2.2. Clinical Sensitivity / Test Agreement Evaluation Analysis

The Positive Percent Agreement (PPA) was defined as the percentage of the positive samples detected by the assay:

PPA = 100* Number of Agreed Positives/Total Number of Positive Samples with Valid Results.

The one-sided lower 95% confidence bound for PPA was determined by the exact (Clopper-Pearson) method.

6.2.3. Acceptance Criteria

Results are descriptive in nature and compared to results provided in Roche's IFU[1] and EUA (EUA200514/A001).

6.2.4. Clinical Sensitivity / Test Agreement Evaluation Results

A summary of the Elecsys anti-SARS-CoV-2 assay testing on COVID-19 (b) (4) serum samples is shown in Table 6. The assay detected the presence of SARS-CoV-2 antibodies in (b) (4) COVID-19 convalescent serum samples with a percent agreement of 93.50%.

Table 6. Positive Agreement in COVID-19 PCR Confirmed (b) (4) Serum Samples

Clinical Performance	Frequency of Elecsys Anti-SARS-CoV-2 Positive Samples	Percent Agreement	Total Samples Tested	95% One-Sided Lower Confidence Limit (%)
Positive Percent Agreement	(b) (4)	93.50	(b) (4)	89.86

7. CONCLUSION

The results documented in this report for the Elecsys SARS-CoV-2 assay indicate the Roche assay was 100% specific when analyzing pre-COVID-19 pandemic samples and 93.5% sensitive in identifying antibodies in samples from subjects (b) (4) days after a COVID-19 PCR diagnosis. The specificity (100%) and sensitivity (93.5%) demonstrated in this report align similarly to Roche's IFU[1] of 99.8% (n=10,453 samples obtained before December 2019; CI 99.69-99.88%) specificity and 99.5% (n=496 samples with a PCR confirmed SARS-CoV-2 infection; CI 97.0-100%) sensitivity in symptomatic patients ≥14 days post-PCR confirmation; sensitivity was 85.3% in samples collected 7-13 days post-PCR confirmation.

In addition, the results of this method validation are similar to what was observed in a previous evaluation of the Elecsys Anti-SARS-CoV-2 assay conducted by Public Health England[4] in which specificity of 100% (CI 99.1-100) and sensitivity of 86.1% (CI 76.5-92.8) was observed using 472 negative samples and 79 convalescent patient samples, respectively. Overall, these data provide evidence that the assay is validated and suitable for its intended use in clinical trials designed to evaluate the efficacy of Pfizer's SARS-CoV-2 vaccine candidate or other epidemiological studies. The assay demonstrated acceptable clinical sensitivity and clinical specificity, comparable to that described in Roche's IFU[1] and EUA (EUA200514/A001), and the evaluation performed at Public Health England.[4]

8. DEVIATIONS

N/A

9. SUPPORTING DOCUMENTATION

1. VR-MVR-10081-ATT01- Supportive Data for VR-MVR-10081 – Validation Data Listing.

10. SUPPLEMENTAL INFORMATION

1. Elecsys Anti-SARS-CoV-2 Instructions For Use. 09203095501, Version 4.0. July 2020. (VR-MVR-10081-Supplemental Info).

2. VR-MVR-10081-ATT02, Supportive Data for VR-MVR-10081 - Roche Correspondence to FDA In Support of Pfizer Method Validation.

3. VR-TM-10304, Test Method for the SARS-CoV-2 Nucleocapsid (N) Antigen Detection Assay.

4. Duggan J. Evaluation of Roche Elecsys Anti-SARS-CoV-2 serology assay for the detection of anti-SARS-CoV-2 antibodies. In: Published 11 Jun 2020. London: Public Health England; 2020: 17 pages.

5. VR-MVP-10075, Protocol for the Method Validation of the Elecsys Anti-SARS-CoV-2 Assay.

6. VR-SOP-LC-11296, Data Review for SARS-CoV-2 Nucleocapsid Antigen Detection Assay.

7. VR-RGR-QC-10753, Quality Control Sample Formulation for Elecsys Anti-SARS-CoV-2 Assay.

8. VR-MVR-10081-ATT03-Supportive Data for VR-MVR-10081 – Roles and Responsibilities for the Method Validation.

9. VR-SOP-FE-11297, Operation and Maintenance of Roche Cobas®(b) (4) analyzer.

10. VR-VPL-10034, Installation and Operational Qualification of Roche Cobas®(b) (4) Analyzer.

11. SUPPORTIVE TABLES

11.1. List of Critical Reagents for the Elecsys SARS-CoV-2 Validation

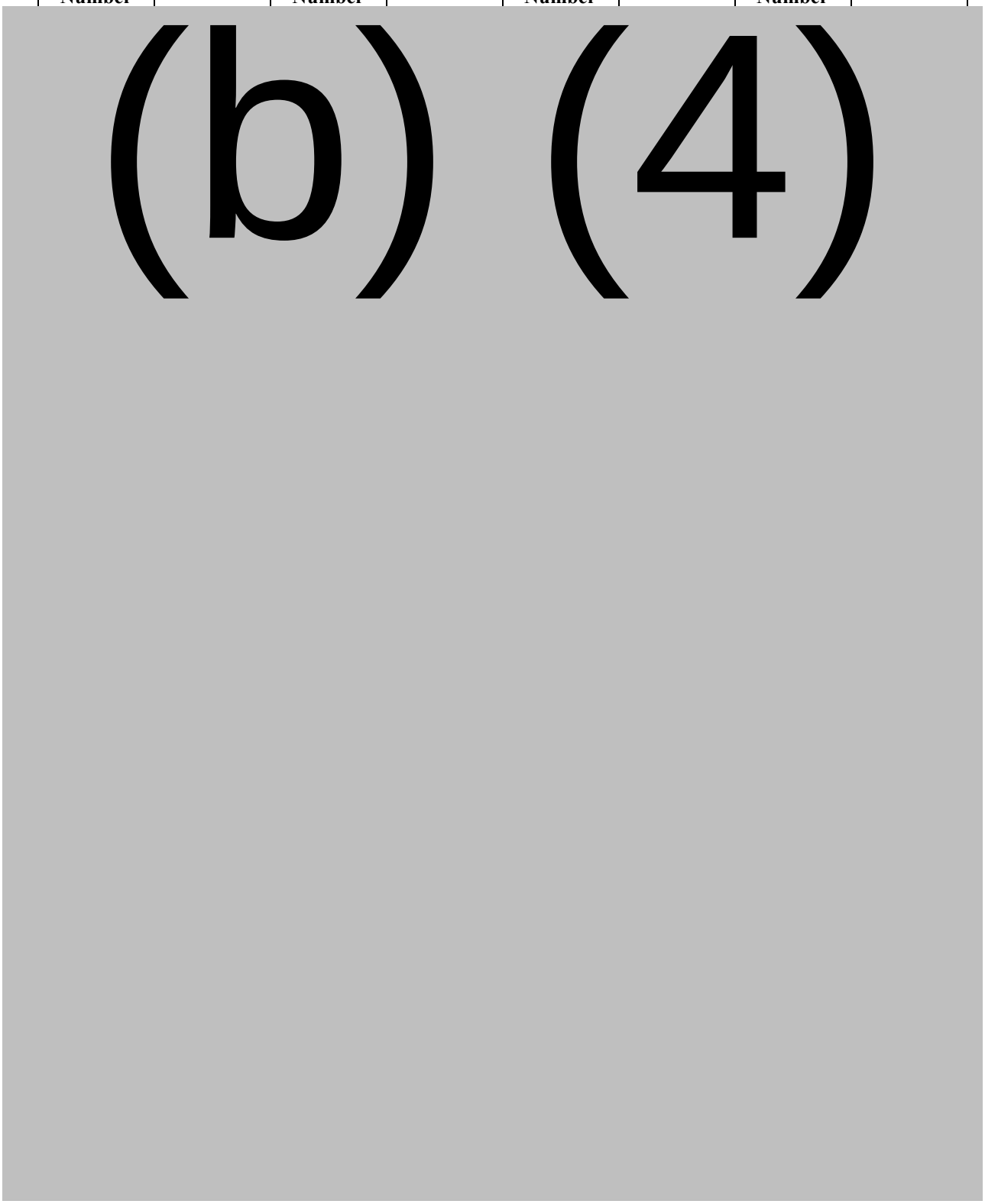
Reagent	Critical Need	Source
R1 SARS-CoV-2-Ag~biotin, (gray cap), 1 bottle, 16 mL: Biotinylated SARS-CoV-2-specific recombinant antigen (*E coli*)	Assay primary reagents	Roche Diagnostics (Indianapolis, IN)
R2 SARS-CoV-2 Ag~Ru(bpy) $_3^{2+}$ (black cap), 1 bottle, 16 mL: SARS-CoV-2-specific recombinant antigen labeled with ruthenium complex	Assay secondary reagents	Roche Diagnostics (Indianapolis, IN)
ACOV2 Cal1 Negative calibrator 1 (white cap), 1 bottle of 0.67 mL: Human serum, non-reactive for anti-SARS-CoV-2 antibodies	Low assay calibrator	Roche Diagnostics (Indianapolis, IN)
ACOV2 Cal2 Positive calibrator 2 (black cap), 1 bottle of 0.67 mL: Human serum, reactive for anti-SARS-CoV-2 antibodies; buffer; preservative	High assay calibrator	Roche Diagnostics (Indianapolis, IN)
Custom Positive Control (b) (4)	Positive Control	(b) (4)
Custom Negative Control (b) (4)	Negative Control	(b) (4)
Serum from SARS-CoV-2 positive donors	Clinical Specificity/Specificity Experiments	(b)(4)
Serum from SARS-CoV-2 negative donors	Clinical Sensitivity/Specificity Experiments	(b)(4) (b) (4)

11.2. Clinical Samples for SARS-CoV-2 Positive Donors

Sample Number	ID	Sample Number	ID	Sample Number	ID	Sample Number	ID
(b) (4)							

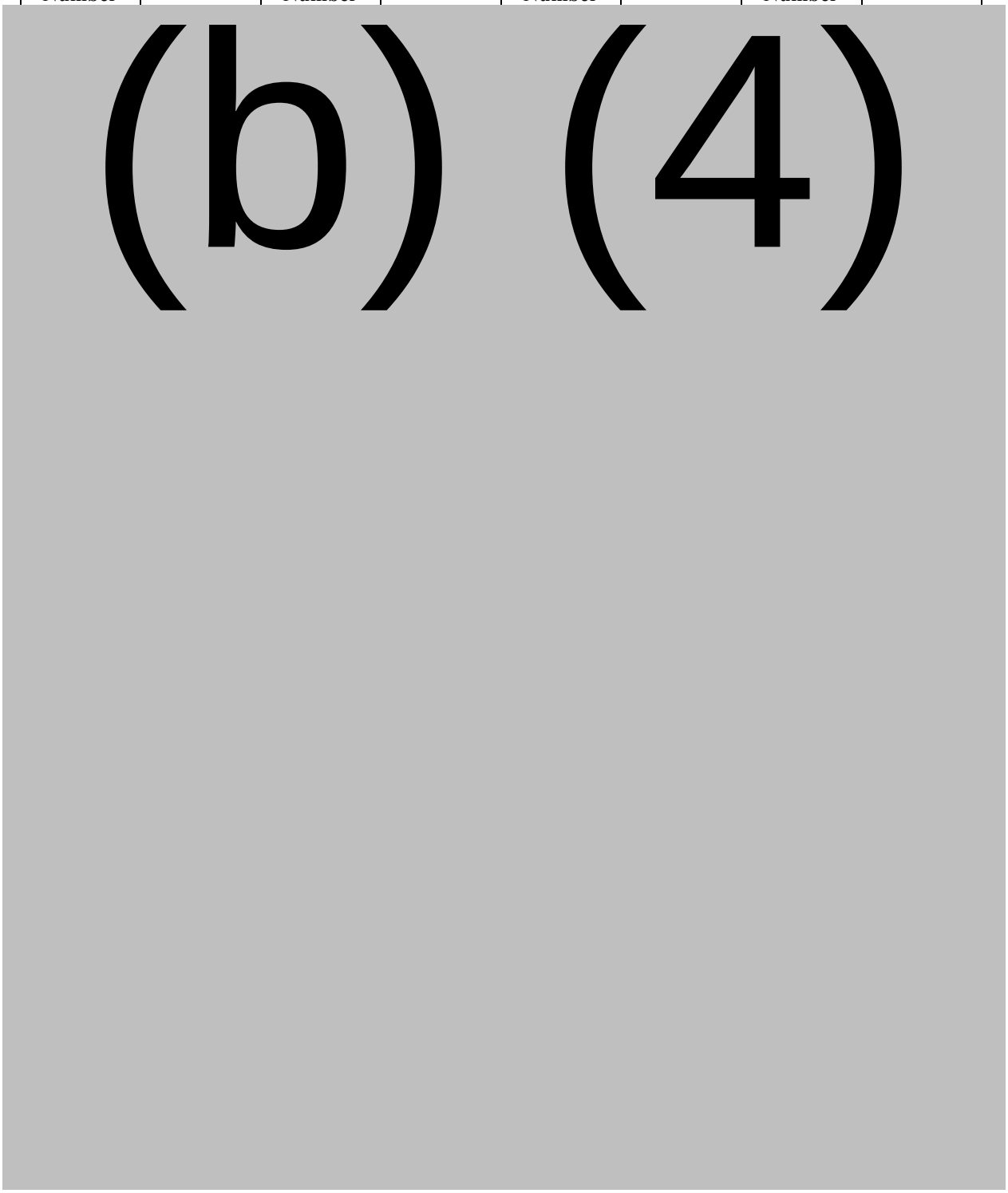
11.2. Clinical Samples for SARS-CoV-2 Positive Donors

Sample Number	ID	Sample Number	ID	Sample Number	ID	Sample Number	ID



11.3. Clinical Samples for SARS-CoV-2 Negative Donors

Sample Number	ID	Sample Number	ID	Sample Number	ID	Sample Number	ID



Document Approval Record

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